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Study of calix[4]resorcinarene–dopamine complexation in mixed phospholipid monolayers formed at the air–water interface

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Abstract

We have studied the physical properties of monolayers formed by calix[4]resorcinarene and in mixtures with dipalmitoyl phosphatidylcholine (DPPC) in various molar ratios formed at the air–water interface and at presence of dopamine in water subphase by means of measurements of surface pressure and dipole potential. We showed that both calix[4]resorcinarene as well as its mixture with DPPC form stable monolayers at the water subphase. The presence of dopamine resulted in an increase of the mean molecular area and in a decrease of the compressibility modulus of the monolayers. For mixed monolayers at higher content of calix[4]resorcinarene (>0.2 molar fraction) a deviation from ideal miscibility took place especially for monolayers in a solid state. This can be connected with formation of aggregates of calix[4] resorcinarene. Lowest miscibility and weakest interaction of dopamine with a monolayer was observed for calix[4]resorcinarene molar fraction of 0.33 in the monolayer.

Keywords: Calix[4]resorcinarene; Monolayer; Surface pressure; Dipole potential; Dopamine; Aggregates

1. Introduction

Calix[n]arenes are macrocyclic aromatic molecules which originate from the synthesis of the phenols and aldehydes, whereas [n] refer to the number of the phenol aromatic cycles in the molecule. In calixarenes, phenolic subunits are bridged via methyl groups in meta position. This provides of the characteristic vase-like shape of the calixarene molecule [1]. In addition, macrocycles with subunits like resorcine or pyrrol have been successfully synthesized [2–4]. Due to the presence of the hydrophobic cavity, formed by the phenolic units, calixarenes are being used for detection of a wide range of compounds such as metal ions, biomacromolecules, etc. Moreover, easy modification of the side groups allows one to prepare tailor-made calixarenes with high affinity for the specific target molecules. Therefore, these recognizing features of calixarenes can be used as a basis for development of biosensors.

Monolayers formed by calix[n]arenes have been studied either on the water subphase [4–7] or on the subphases containing various cations [1,8–12]. In addition, calix[4]arenes monolayers have been also successfully employed for the detection of monomeric nucleosides in the subphase [13–15]. Recently, considerable attention was focused on the development of the methods of detection of the neurotransmitters adrenaline, dopamine or ephedrine, which trigger the metabolism of the lipids and sugars, followed by their utilization in the energetic processes. Uncontrolled imposition of these catecholamines acting as the doping and their difficult detection has provoked International Olympic Federation into the search for the rapid and foolproof methods, which could estimate the concentrations of the catecholamines in the urine. Synthetic calix[4]resorcinarene was extensively studied in this respect. It has been shown, that the biosensor based on the solid supported lipid membranes with incorporated calix[4]resorcinarene is highly sensitive towards catecholamines with detection limit in μM concentration range [16–22]. These sensors were selective and did not reveal any significant interferences with other compounds (e.g. ascorbic acid, lactose, urea, etc.) [16–22]. Despite these extensive studies, the mechanisms of interaction of catecholamines with calix[4]resorcinarene and the mechanisms of interaction of this artificial receptor with phospholipids

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is still under investigations. The study of the properties of calix[4]resorcinarene monolayers and their mixtures with phospholipids has also valuable impact for the development biosensors based on mass detection methods, e.g. quartz crystal microbalance. The monolayers formed by calixarenes can be deposited on a surface of quartz crystal covered by hydrophobic film, e.g. alkanethiols or stearic acid. Thus, due to hydrophobicity of the stearic acid the possible interferences of polar molecules, e.g. dopamine with a supporting layer should be minimized. Our preliminary results using Langmuir–Blodgett technique proved the possibility of the formation a stable calix[4]resorcinarene film on a surface of stearic acid adsorbed on a SiO_2 support.

In this work we applied Langmuir film technique and dipole potential measurements for studying the thermodynamical, mechanical and electrical properties of the monolayers formed by calix[4]resorcinarene sensitive to dopamine. We studied the properties of the monolayers formed exclusively by this calix[4] resorcinarene, but also the properties of mixed calix[4] resorcinarene–phospholipid monolayers formed at the water subphase or subphase containing various concentrations of dopamine. The binding of dopamine is shown to change the shape of the surface pressure isotherms, as well as changes of the dipole potential, which indicates the charge redistribution upon the dopamine binding, as well as the reorganization of the molecular structure of the monolayers. We also showed that depending on molar ratio between phospholipids and calix[4] resorcinarene the aggregates of this calixarene can also be formed.

2. Materials and methods

2.1. Chemicals and formation of monolayers

Calix[4]resorcinarene was synthesized according to the method published in detail elsewhere [19,21]. In our experiments we used dipalmitoyl phosphatidylcholine (DPPC) and dopamine (Sigma, USA). The monolayers were prepared by spreading of a small amount (25–30 μl) of the chloroform solution (p.a. grade) (Slavus, Slovakia) of calix[4]resorcinarene or DPPC/calix[4] resorcinarene mixtures (concentration 1 mg/ml) using a microsyringe (Cosge, Australia) either on the water subphase of the Langmuir trough (resistance >15 MΩ cm, ELIX 5, Millipore, El Paso, USA) or on the water subphase containing dopamine at the final concentration in the range 10–1000 μM. The DPPC/calix[4]resorcinarene mixtures were prepared at the molar ratios of 10:1, 5:1, 3:1 and 1:1. Each monolayer was allowed to equilibrate for 15 min. This time was sufficient for solvent evaporation.

Surface pressure–area (π–A) isotherms were measured using computer-controlled Langmuir trough 311D (NIMA Technology, Coventry, UK) made of Teflon (volume ~230 ml, the whole surface area was 300 cm^2) and Teflon-bar barrier. The through was equipped with Wilhelmy plate pressure sensor NIMA PS4. All monolayers were compressed at the constant speed of 5 cm^2/min. The temperature of the subphase was maintained constant by thermostat Lauda RE206 (Königshofen, Germany).

2.2. Dipole potential

The dipole potential ΔV of the monolayer is difference between the potential of a monolayer and that of pure subphase. The measurement of dipole potential allows to analyze changes in the orientation of the molecular dipoles in the monolayer during compression. Presence of the monolayer between electrodes causes changes of the potential based on the Helmholtz equation:

$$\Delta V = \mu_n/(A \cdot \varepsilon_r \cdot \varepsilon_o) \quad (1)$$

where ε_r and ε_o are the relative dielectric constant and the permittivity of vacuum, respectively, μ_n is the normal component of the dipole moment of the molecule and A is the molecular area. The dipole potential was measured by means of vibrating plate method [23] using high sensitive electrostatic voltmeter 320 C and electrode 3250 (Kelvin probe) (TREK Inc., USA) mounted on special adjustable platform (NIMA). The probe was situated in the air approximately 1.5 mm above the surface. The dipole potential was measured in respect to the pure subphase. The potential of later was taken as zero. Dipole potential was measured simultaneously with the surface pressure using NIMA interface and software.

The accuracy of measurement the surface pressure and dipole potential was 1 mN/m and 1 mV, respectively. All experiments were performed at 24 °C and repeated at least six times to ensure the reproducibility of the results.

3. Results and discussion

The structure of calix[4]resorcinarene is shown in Fig. 1. Due to the presence of the four hydrophobic aliphatic hydrocarbon chains calix[4]resorcinarene forms stable monolayers on the water subphase. The pressure–area (π–A) isotherm and dipole potential- area plots of monolayer formed by calix[4]resorcinarene at water subphase are presented in Fig. 2. The π–A isotherm is characterized by a long part at the constant pressure ~0 mN/m (areas higher than 1.6 nm^2/molecule). The compression resulted in appearance of small, almost negligible inflection at ~5 mN/m. The surface pressure starts to grow practically linearly at areas >1.6 nm^2/molecule. The collapse of the monolayer takes place at approximately 40 mN/m. The results agree well with paper by Volhardt et al. [6] that studied the properties of the calix[4]arene monolayers of similar structure and showed that at temperatures <26 °C the monolayers are in condensed phase already at surface pressure close to 0. The dipole potential starts to grow at ~1.8 nm^2/molecule. This is probably due to reorientation of the calix[4] resorcinarene molecules, mainly of their hydrocarbon chains. The dipole potential reaches maximum (~125 mV) at the region corresponding to inflection point at π–A isotherm (at surface pressure of 5 mN/m). This suggests well ordered monolayer already at relatively low surface pressure. Using the Eq. (1) it is possible to estimate the dipole moment of calix[4]resorcinarene. At the maximum value of dipole potential, the surface dipole

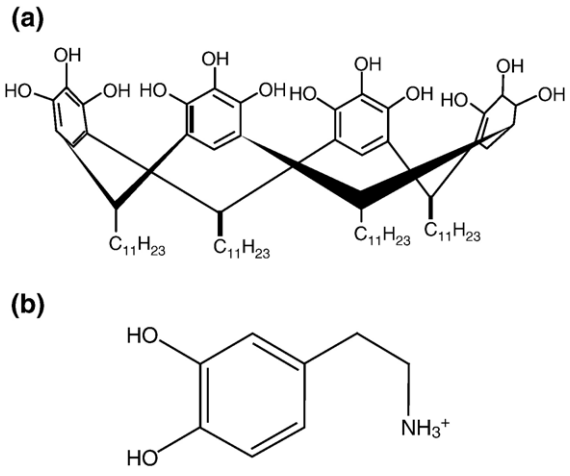

Fig. 1. Chemical structures of (a) calix[4]resorcinarene and (b) dopamine.

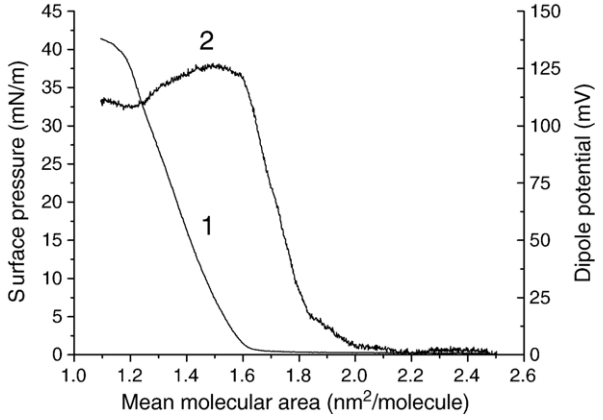

Fig. 2. The plot of (1) surface pressure and (2) dipole potential as a function of mean molecular area for monolayers of pure calix[4]resorcinarene at water subphase.

moment is approximately 0.5 D. Consecutive compression causes only an approach of molecules closer to each other, followed by an alteration of orientation of the molecules, as this can be seen from the decrease of the surface potential at the lower areas to the final value of ~110 mV at the collapse of the monolayer. The value of the surface potential of the calix[4] resorcinarene monolayer agrees with the values obtained in Ref. [24] that studied similar calix[4]arene monolayers using various buffer solutions. Extrapolation of the linear part of the isotherm corresponding to the solid state of the monolayer to the zero surface pressure allow us to estimate molecular area of the calix [4]resorcinarene molecule, which is approximately 1.5 nm^2/ molecule. This value matches well the mean molecular areas of the calix[4]arenes of similar structure obtained by other authors [4,6,7].

In order to study the interaction of dopamine with calix[4] resorcinarene the monolayers of calix[4]resorcinarene were formed on the subphases containing various concentrations of dopamine. As it can be seen from Fig. 3a, increasing concentration of dopamine produces a shift of the isotherms toward higher areas. This effect is accompanied by an increase of the mean molecular area of calix[4]resorcinarene. The values of the mean molecular areas are reported in Table 1. As it can be seen in Table 1, the mean molecular area of calix[4] resorcinarene increases at the presence of 1 mM of dopamine by approximately 7% (the differences between the molecular area without dopamine and at certain dopamine concentration were statistically significant according to the Student's t test ($P<0.001$)). The plot of mean molecular area as a function of dopamine concentration represents a typical Langmuir isotherm (Fig. 3b). There is tendency to saturation at higher concentrations of dopamine and the interaction between dopamine molecules and the receptors is much stronger than those between dopamine molecules at the surface of the monolayer (see [25] for theory of specific adsorption). The dissociation constant, K_D was determined according to equation $A=A_{max}.c/(K_D+c)$, where A_{max} is the maximal molecular area and c is the dopamine concentration and was found ca. 310 nM. This is much higher in comparison with e.g. the binding affinity of

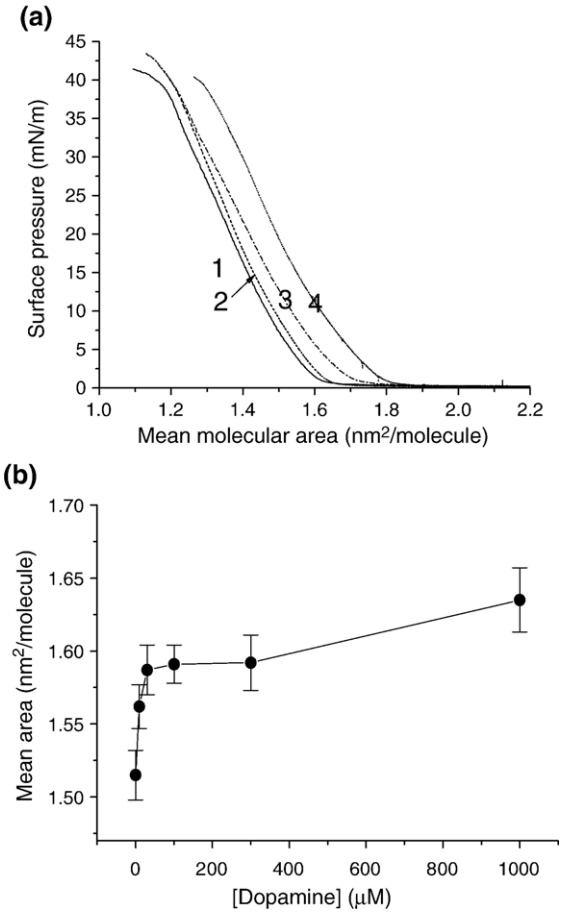

Fig. 3. (a) The plot of surface pressure as a function of mean molecular area for monolayers of calix[4]resorcinarene at water subphase (1) and those contained dopamine in concentration: (2) 10; (3) 100 and (4) 1000 μM, respectively. (b) The plot of mean molecular area as a function of dopamineconcentration for monolayers composed of calix[4]resorcinarene formed on water subphase.

antibodies or DNA aptamers, which are in the range of 1–100 nM [26].

In order to check specificity of the binding of dopamine, we also studied the interactions of dopamine with the DPPC monolayers without receptors. Fig. 4a shows a plot of the monolayers formed by DPPC at the water subphase without dopamine and at various dopamine concentrations. DPPC isotherm posses well-defined plateau localized at ~10 mN/m,

Table 1
Mean molecular area, A, compressibility modulus, C_S^{-1}, and the excess Gibbs energy, ΔG^{ex}, for the monolayers of calix[4]resorcinarene formed at pure water subphase and those contained various concentration of dopamine

Dopamine,μM	A (nm^2/molecule)	C_S^{-1} (mN/m)	ΔG^{ex} (kJ/mol)
0	1.52±0.02	178.4±4.1	0
10	1.56±0.02	176.7±4.0	1.65±0.79
100	1.59±0.01	171.2±5.5	2.66±0.75
1000	1.64±0.02	165.2±5.3	4.20±0.97

The upper limit value of surface pressure used in calculation of ΔG^{ex} according to Eq. (3) was 35 mN/m. Results represent mean±SD determined from 6 independent experiments. The differences between mean molecular areas are statistically significant according to the Student's t test ($P<0.001$).

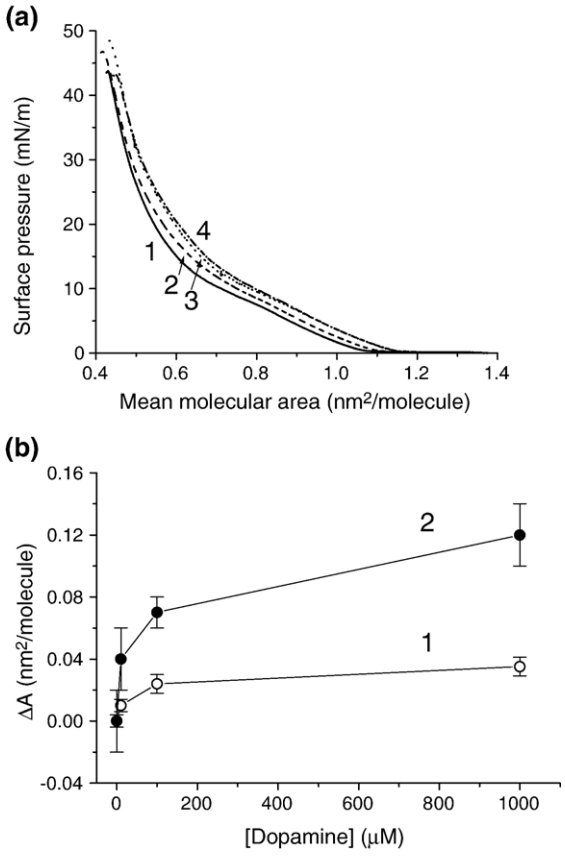

Fig. 4. (a) The plot of surface pressure as a function of mean molecular area for monolayers of DPPC at water subphase (1) and those contained dopamine in concentration: (2) 10; (3) 100 and (4) 1000 μM, respectively. (b) The plot of the changes of mean molecular area ΔA ($\Delta A = A - A_0$, where A_0 is the mean molecular area without dopamine and A those at certain dopamine concentration) as a function of dopamine concentration for monolayers composed of 1—DPPC and 2 calix[4]resorcinarene formed on the water subphase.

which corresponds to the transition from the liquid-expanded (L-E) to the liquid-condensed (L-C) phase and collapses at ~50 mN/m (it should be noted that the collapse pressure depends particularly on the level of the subphase in a through as well as on the speed of the compression. The obtained value of collapse may be due to a leakage of the monolayer through the barrier at higher pressures. Therefore, we analyzed the properties of the DPPC monolayers at pressures far below the collapse pressure). As it is seen in Fig. 4a, the presence of dopamine also affects the π–A isotherms that shift towards higher areas with an increase of the concentration of dopamine in the subphase. In order to compare the effect of dopamine on the mean molecular area of calix[4]resorcinarene and of DPPC, we constructed a plot of the changes of the mean molecular area as a function of dopamine concentration (Fig. 4b). It can be seen in Fig. 4b, the effect of dopamine on the increase of the molecular area of calix[4]resorcinarene and of DPPC is comparable at relatively low dopamine concentrations (10 μM). However, at higher concentrations the presence of dopamine resulted in larger changes of the mean molecular area of calix[4] resorcinarene in comparison with those of DPPC.

More detailed information about the physical properties of the monolayer can be achieved from the compressibility modulus, C_S^{-1}, defined as [11]:

$$C_S^{-1} = -A\left(\frac{\delta\pi}{\delta A}\right) \tag{2}$$

According to Ref. [11], compressibility modulus values between 0 to 12.5 mN/m refer to the gas phase of the films, from 12.5 to 50 mN/m to the liquid-expanded (L-E) films, from 100 to 250 mN/m to the liquid - condensed (L-C) films and the values above 250 mN/m are typical for the solid films. Using Eq. (1), we determined the compressibility modulus for condensed state of the monolayers. The quantity $\left(\frac{\delta\pi}{\delta A}\right)$ is the slope of the monolayer and the area A corresponds to the mean molecular area at condensed state of the monolayer (Table 1). As it can be seen from the Table 1, all the investigated monolayers are characterized by compressibility modulus > 165 mN/m. This proves the condensed state of the monolayers. It is also seen in Table 1 that with increasing of dopamine concentration, the values of C_S^{-1} tend to decrease (although the statistical analysis using Student's t test proved the significant differences ($P < 0.001$) between the C_S^{-1} values only at 0 and 1000 μM of dopamine). This suggests that changes of the conformation of the calix[4] resorcinarene due to binding of dopamine caused more flexible (i.e. more compressible) monolayer.

Thermodynamic stability of the monolayer formed on dopamine-containing subphase with the respect to the water subphase can be determined by analysis of the excess of the Gibbs free energy. The excess of Gibbs free energy, ΔG, can be evaluated by the following equation [11]:

$$\Delta G = \int_0^{\pi} A_{\text{dopamine}} d\pi - \int_0^{\pi} A_{\text{water}} d\pi \tag{3}$$

where π is the upper limit pressure and was chosen to be 35 mN/m, i.e. below the collapse of the monolayer. When dopamine is present in the subphase, the ΔG values are positive. This indicates destabilization of the monolayers. The binding of dopamine into the calix[4]resorcinarene cavities probably causes change of the structure of the calix[4]resorcinarene molecules, which is accompanied by the weakening of the forces acting between the adjacent calix[4]resorcinarene molecules in the monolayer. A comparison of ΔG values in Table 1 shows that an increase of concentration of dopamine causes an increase of the excess Gibbs energy. This confirms the hypothesis of complexation between the calix[4]resorcinarene and dopamine even at the relatively low concentration (10 μM).

Measurements of dipole potential have revealed influence of the dopamine on the calix[4]resorcinarene monolayer upon compression, mainly in low pressure region. This can be seen in Fig. 5a where a plot of the dipole potential as a function of mean molecular area is presented for monolayers of calix[4]resorcinarene at water subphase and those containing dopamine at various concentrations. As it is seen in Fig. 5a an increase of dopamine concentration causes a shift of the potential-area dependencies to higher dipole potentials at low pressure region. However at higher surface pressure and at presence of dopamine,

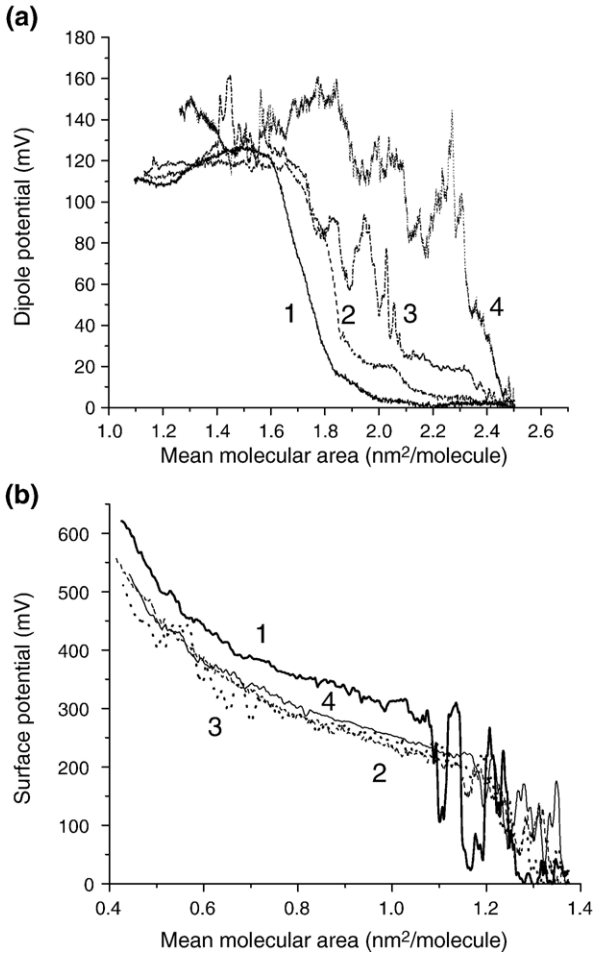

Fig. 5. The plot of dipole potential as a function of mean molecular area for monolayers composed of (a) calix[4]resorcinarene and (b) DPPC at water subphase (1) and those contained dopamine in concentration: (2) 10; (3) 100 and (4) 1000 μM, respectively.

the dipole potentials are higher than those without dopamine (~120–130 mV). The tendency of the dipole potentials to grow since the very beginning of the compression can be related to the binding of dopamine to the monolayer. The pK_a value of dopamine in a water subphase is 8.87 [27]. Therefore at pH of water subphase used in experiments (pH ≈ 6), the amino group of dopamine (Fig. 1b) is positively charged and has affinity to the –OH groups of the calix[4]resorcinarene molecules exposed into the water subphase. This pK_a value could in principle shift down when dopamine bind to a monolayer. However, despite this possibility we suppose that the electrostatic interactions between dopamine and the calix[4]resorcinarene hydroxyl groups are the main reason of the changes of dipole potential. From Fig. 5a, we can also see that before the main raise of the dipole potential certain irregularity took place at ~2.1 nm^2/molecule. The magnitude of this irregularity increases with increasing the dopamine concentration. This effect may be associated with changes of the calix[4]resorcinarene structure due to dopamine binding. We also studied the effect of dopamine on the dipole potential of the monolayers of DPPC without calix[4]resorcinarene. Fig. 5b shows the plot of the dipole potential of these monolayers without dopamine and at various dopamine concentrations at the subphase. For potential-area plot corresponding to pure DPPC one can see that the increase of the dipole potential starts at relatively high mean molecular area (~1.4 nm^2), when the monolayer is in gaseous state. The molecules start to orient themselves when the normal component of the dipole moment increases (curve 1, Fig. 5b). The abrupt stop of the potential increase occurs at ~1.1 nm^2, when the surface pressure starts to grow (curve 1, Fig. 4a); between areas 1.1–0.7 nm^2 only a slow rise of the dipole potential takes place. This region includes also the transition of the monolayer from L-E to L-C state. We can conclude that in this state the molecules are just getting closer each to another and their tilt angle changes only slightly. Another sharper increase of the surface potential can be observed at mean molecular area between 0.7 nm^2 and 0.58 nm^2. Some structural reorganization of the monolayer occurs that causes not only transition from L-E to L-C state, but also change of the orientation of the molecules. Transition from L-C to solid (S) state at molecular area above 0.58 nm^2 is characterized by another raise of the dipole potential. Maximal value of dipole potential in S state of DPPC monolayer was 617 mV. This value is in good agreement with those reported by Shapovalov et al. [28]. The value of 617 mV corresponds to dipole moment of polar head group of DPPC and according to Eq. (1) it is approximately 0.65 D. From comparison of dipole moments of DPPC and resorcin[4]arene it is evident that DPPC is characterized by only slightly (1.3 times) higher dipole moment than calixarene. In contrast with the effect of dopamine on the dipole potential of calix[4] resorcinarene monolayers, the monolayers composed of DPPC revealed rather weak sensitivity to dopamine. Taking into account that the DPPC monolayer is in general neutral, it is not surprising that the interaction of dopamine with these monolayers should be weaker then those for negatively charged resorcin[4]arene monolayers.

The surface pressure isotherms for mixed DPPC/calix[4] resorcinarene monolayers formed on the water subphase without dopamine are presented in Fig. 6a. DPPC, calix[4]resorcinarene and their mixtures of various molar ratios produce stable monolayers. The properties of the pure calix[4]resorcinarene and DPPC monolayer have been previously discussed. Isotherms of the mixed monolayers reveal the shapes, which are superposition of the isotherms of the both pure components. However, plateau region is markedly reduced even at the molar ratio DPPC/calix[4]resorcinarene of about 10:1 and becomes almost negligible at the molar ratio 3:1. At the later molar ratio, the shape of isotherm is similar to those of pure calixarene, although the number of DPPC molecules is 3 times higher than that of the calix[4]resorcinarene molecules. Values of the mean molecular areas, obtained by the extrapolation of the isotherm corresponding to the solid phase to the zero surface pressure, and compressibility modulus of these monolayers are presented in Table 2. As it can be seen in this table, an increase of the content of calixarene in DPPC results in an increase of the mean molecular area and in a decrease of the compressibility modulus. Thus, pure DPPC monolayer is less compressible in comparison with pure calixarene monolayer.

Dipole potentials of all the monolayers (pure and mixed) were recorded in parallel with surface pressures and are

presented in Fig. 6b. The peculiarities of potential-area plot corresponding to pure DPPC has been discussed above. For mixed monolayers, we observed a decrease of the maximal value of dipole potential with an increase of the content of calix[4]resorcinarene in monolayers. Moreover, the abrupt growth of the dipole potential observed for DPPC monolayer at ~0.7 nm^2/molecule is diminished with an increase of the calix[4]resorcinarene molar fraction in the monolayer and shifts to lower molecular area. Thus, both DPPC and calix[4]resorcinarene affect the dipole potential of the monolayer. A more detailed insight into the miscibility of monolayer components can be obtained by calculating the excess area as compared to the ideal mixing. For a given surface pressure the expression for excess area is according to [29] given by equation:

$$A_{12} = XA_1 + (1-X)A_2 \quad (4)$$

where A_1 and A_2 are the molecular areas of calix[4]resorcinarene and DPPC, respectively. A_{12} is the average area per molecule of the mixed monolayer and X is the molar fraction of calix[4]resorcinarene. In the case of ideal miscibility, the plot of A_{12} vs. X should be straight line connecting the values of mean molecular areas of pure components. Negative deviation from ideal miscibility indicates interaction between molecules, e.g. formation of DPPC/calix[4]resorcinarene complexes, while positive deviation can be related to formation of aggregates of pure components [29]. In order to analyze the properties of calix[4]resorcinarene in a monolayers we constructed the plot of the mean molecular area as a function of molar fraction of calix[4]resorcinarene for three different surface pressures: 5, 15 and 35 mN/m (below the DPPC plateau, just above plateau and at the solid phase). These plots are presented in Fig. 7a. As it is seen in Fig. 7a, a negative deviation from the ideality appears at 5 mN/m at all molar fraction of calix[4]resorcinarene, whereas at 15 and 35 mN/m a positive deviation from ideality was observed. A negative deviation from ideality indicates presence of the attractive forces between the molecules of both types, while a positive deviation suggests the mutual immiscibility of both components, i.e. formation of aggregates of pure components. The negative deviations at 5 mN/m can be caused by the non-ordered monolayer with the molecules distributed randomly on the surface with no tendency to either aggregate or to mix mutually. Therefore, it would not be correct to speculate any miscibility below the phase transition, although the molecules are relatively well oriented, as it can be seen from the onset of the surface potentials even in the gas phases of the monolayers. Positive deviations from the ideality at 15 mN/m indicate some immiscibility of both the components and formation of the domains of the molecules of one type. On the other hand, at low molar fractions of calix[4]resorcinarene the phospholipid and calix[4]resorcinarene molecules are miscible. When the monolayers are in solid state and below the collapse (at surface pressure 35 mN/m), it seems that the miscibilities do not differ too much from these at 15 mN/m, although almost negligible negative deviations appear at 10:1 and 5:1 DPPC/calix[4]resorcinarene molar ratios.

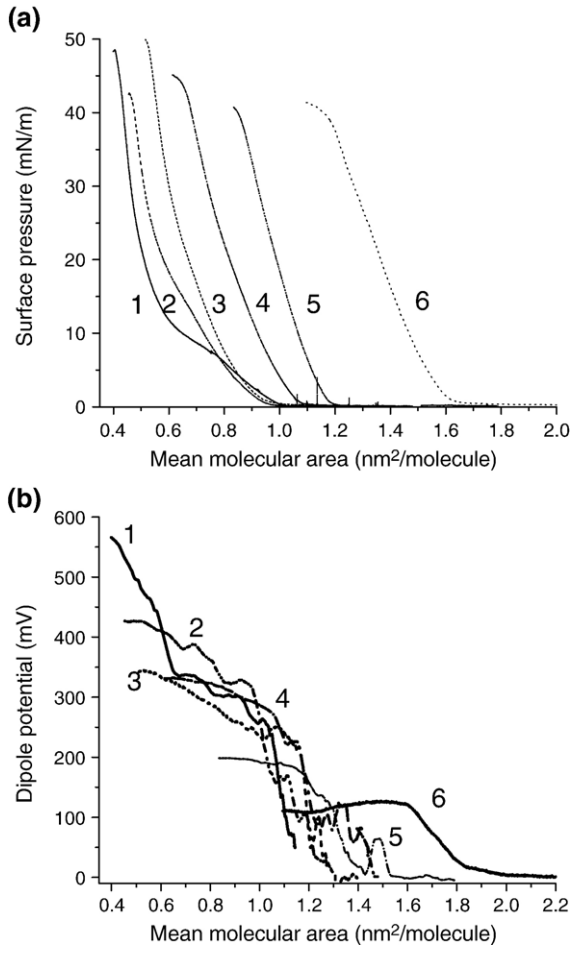

Fig. 6. The plot of (a) surface pressure and (b) dipole potential as a function of mean molecular area for mixed monolayers composed of DPPC and calix[4]resorcinarene of different molar ratios at water subphase: DPPC/calix[4]resorcinarene = 1:0 (1) (pure DPPC); 10:1 (2); 5:1 (3); 3:1 (4); 1:1 (4) and 0:1 (6) (pure calix[4]resorcinarene).

Table 2
Mean molecular area, A, and compressibility modulus, C_S^{-1}, for the pure DPPC and calix[4]resorcinarene monolayers and for their mixtures at various molar ratios formed on the water subphase

Molar ratio DPPC: Calix[4]resorcinarene	Mean molecular area A (nm^2/molecule)	Compressibility modulus C_S^{-1} (mN/m)
1:0	0.54±0.01	197.8±5.5
10:1	0.61±0.01	191.5±6.6
5:1	0.72±0.1	189.5±2.8
3:1	0.91±0.01	165.1±4.2
1:1	1.12±0.01	178.2±2.7
0:1	1.52±0.02	176.4±8.1

Results represent mean±SD determined from 6 independent experiments. The differences between mean molecular areas are statistically significant according to the Student's t test ($P<0.001$).

Further insight on the interaction of calix[4]resorcinarene with DPPC can be obtained by analysis of the excess Gibbs energy, ΔG^{ex} [29]:

$$\Delta G^{ex} = \int_{\pi^0}^{\pi} A_{12}\mathrm{d}\pi - X\int_{\pi^0}^{\pi} A_1\mathrm{d}\pi - (1-X)\int_{\pi^0}^{\pi} A_2\mathrm{d}\pi \quad (5)$$

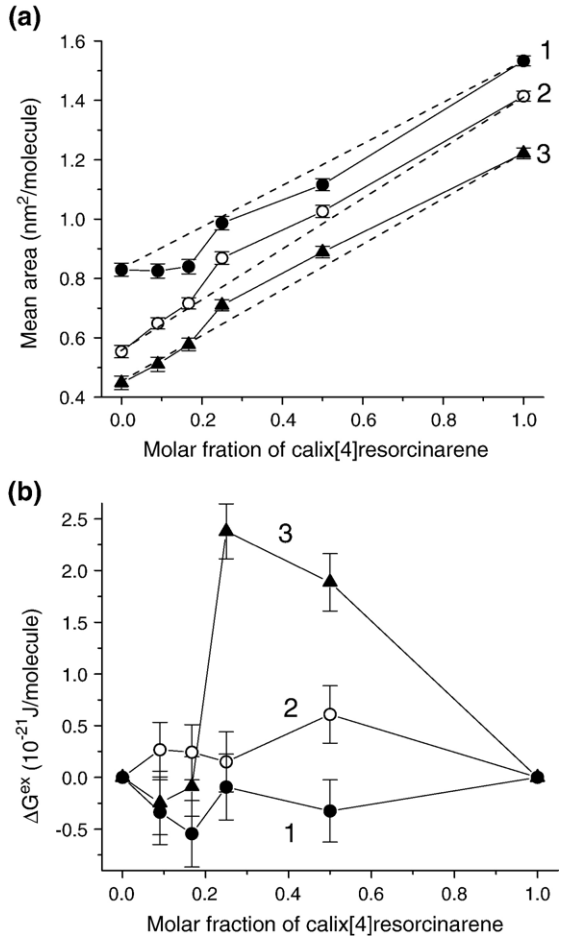

Fig. 7. The plot of (a) mean molecular area and (b) excess free energy, ΔG^{ex}, versus molar fraction of calix[4]resorcinarene in the DPPC monolayers calculated according to Eqs. (4) and (5), respectively for three values of surface pressures: ●—5; ○—15; ▲—35 mN/m. Monolayers were formed on pure water subphase. Results are mean±SD determined from 6 independent measurements.

where A_{12}, A_1, A_2 and X are as previously defined and π is upper limit of surface pressure. The lower limit of integration corresponds to $\pi=0$, while upper limit can be arbitrary selected (we selected $\pi=5$, 15 and 35 mN/m). The value of ΔG provides information whether the particular interaction is energetically favorable ($\Delta G<0$) or not ($\Delta G>0$), while for $\Delta G=0$ ideal mixing takes place. The plots of ΔG^{ex} as a function of molar fraction of calix[4]resorcinarene for three surface pressures are presented in Fig. 7b. Similarly the same conclusions like those for excess areas are valid for Gibbs energies of mixing. A negative deviations from ideality at all ratios at 5 mN/m and at low molar ratios of calix[4]resorcinarene in the monolayer at all surface pressures indicate that mixing is more favorable rather than separation between the two species. At higher surface pressures (15 and 35 mN/m) and at higher molar fraction of calix[4]resorcinarene (>0.3) the phase separation took place between the monolayer components. The phase separation between calix[4]resorcinarene and DPPC is not surprising considering well-ordered DPPC monolayers at higher surface pressures. It is likely that phase separation could be minimized or even disappear for less ordered phospholipid monolayers composed of e.g. unsaturated hydrocarbon chains.

Presence of dopamine at the concentration of 100 μM had noticeable effect on the properties of the mixed monolayers, similar to the effect of dopamine on pure calix[4]resorcinarene monolayers as it was previously described. The plot of surface pressure as a function of mean molecular area for pure components and for mixed monolayers at various molar ratios of DPPC/calix[4]resorcinarene at presence of 100 μM dopamine at water subphase is shown in Fig. 8a. It is seen from this figure, that surface pressure of all the isotherms start to increase at relatively higher areas and reveal approximately the same critical pressure values ~40 mN/m. For pure DPPC and mixed DPPC/calix[4]resorcinarene monolayers formed at water subphase contained 100 μM dopamine, we observed increase in the mean molecular areas in comparison with pure water subphase. The results of determination of mean molecular area, compressibility modulus and excess Gibbs energy are summarized in Table 3. The differences between compressibility modulus of

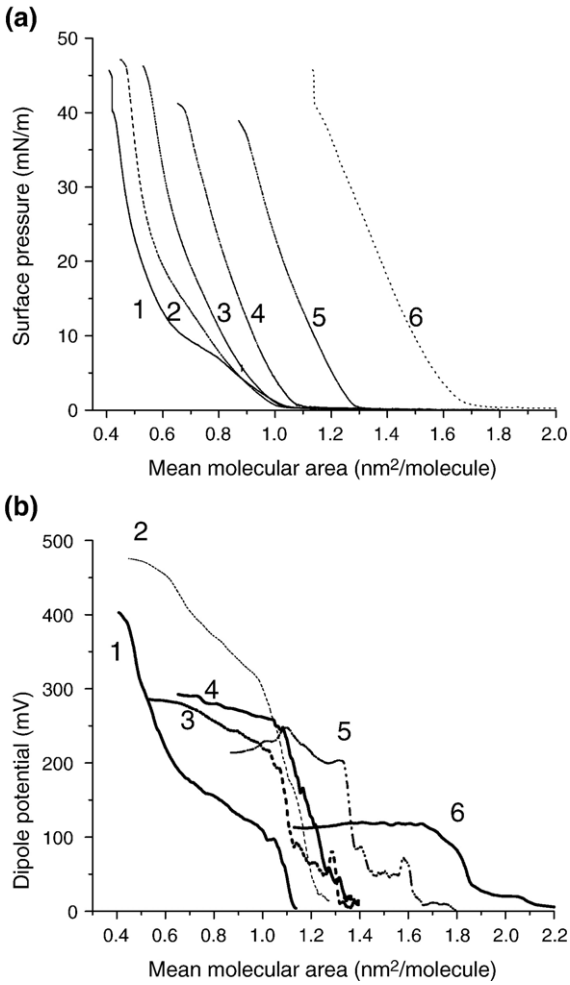

Fig. 8. The plot of (a) surface pressure and (b) dipole potential as a function of mean molecular area for mixed monolayers composed of DPPC and calix[4] resorcinarene of different molar ratios at water subphase contained 10^{-4} M dopamine: DPPC/calix[4]resorcinarene=1:0 (1) (pure DPPC); 10:1 (2); 5:1 (3); 3:1 (4); 1:1 (4) and 0:1 (6) (pure calix[4]resorcinarene).

Table 3
Mean molecular area, A, compressibility modulus, C_S^{-1}, and excess Gibbs energy, ΔG^{ex}, values for the pure calix[4]resorcinarene and mixed DPPC/calix[4]resorcinarene monolayers formed on the water subphase containing 100 μM dopamine

Molar ratio DPPC: Calix[4]resorcinarene	A (nm^2/molecule)	C_S^{-1} (mN/m)	ΔG^{ex} (kJ/mol)
1:0	0.55±0.01	196.9±5.8	0.43±0.21
10:1	0.62±0.01	193.2±6.8	0.42±0.31
5:1	0.74±0.01	187.2±4.1	0.82±0.44
3:1	0.92±0.01	150.5±1.9	0.11±0.41
1:1	1.16±0.01	176.8±8.9	1.05±0.47
0:1	1.59±0.01	171.2±5.5	2.66±0.75

The upper limit value of surface pressure used in calculation of ΔG^{ex} according to Eq (3) was 35 mN/m. Results represent mean±SD determined from 6 independent experiments. The differences between mean molecular areas are statistically significant according to the Student's t test ($P<0.001$).

monolayers formed at water and dopamine contained subphase are not statistically significant. On the other side, binding of dopamine resulted in changes of thermodynamic properties of the monolayers as it can be seen from the changes of Gibbs free energies regarding to the pure water subphase calculated according to Eq (3) (see Table 3). As it can be seen in this table, the changes of Gibbs free energy increase with an increase of the molar fraction of the calix[4]resorcinarene in the monolayer. This effect can be related to the binding of dopamine inside of the calix[4]resorcinarene cavities. It is clearly seen that pure calix[4]resorcinarene monolayer is most sensitive to the presence of the dopamine, since the changes of Gibbs free energy is the highest. The monolayers with the minor content of calix[4]resorcinarene are less sensitive towards dopamine and even at a ratio 1:1 the value of ΔG is 2.5 times lower in comparison with pure calix[4]resorcinarene monolayers. One has to notice that monolayers of DPPC/calix[4]resorcinarene ratio 3:1 were characterized by much lower value of compressibility modulus comparing to the other monolayers and too low difference of the Gibbs energy. The reason of this effect maybe explained as follows. We assume that the weak detection ability for DPPC/calix[4]resorcinarene molar ratio 3:1 is caused by some disfunctional orientation of calix[4]resorcinarene cavities. This effect correlates with biggest deviations from ideality for both excess area and Gibbs energy of mixing on the water subphase and dopamine subphase (see Figs. 7a, b and 9a, b), that evidence on rather inhomogeneous structure of monolayer.

The plot of dipole potential vs. mean molecular area for pure and mixed DPPC/calix[4]resorcinarene is shown in Fig. 8b. These dependencies exhibit similar behavior like those on the pure water subphase. In the case of the pure DPPC, we observed a decrease of the maximal value of dipole potential from 560 mV without dopamine to 400 mV at presence of 100 μM dopamine. A small onset and small offset of the potential were detected for 10:1 and 5:1 ratios, respectively. In the case of molar ration of DPPC/calix[4]resorcinarene of ca. 1:3, we also observed decrease of dipole potential at presence of dopamine (from 320 mV without dopamine to 290 mV at presence of dopamine). Different situation was observed for 1:1 molar ratio. The dipole potential increases from 200 to 230 mV at presence of dopamine. The effect of dopamine on the monolayers composed of pure calix[4]resorcinarene monolayer was previously presented.

As it was already discussed, the monolayers composed of DPPC and calix[4]resorcinarene at 3:1 molar ratio exhibits the highest positive deviations of the excess area (Figs. 7a, 9a) and Gibbs free energy (Figs. 7b, 9b) at the higher surface pressures without and at presence of dopamine. Moreover, tendency of the Gibbs free energy to acquire positive deviations is observed at almost all molar ratios at the surface pressures of 15 and 35 mN/m. One can suggest that complexation between calix[4]resorcinarene and DPPC that occur during compression destabilizes the planar forces acting between the adjacent molecules in the subphase. Such effect seems to be dependent on the molar fraction of calix[4]resorcinarene. It has been already shown, that monolayers formed on the pure water and at DPPC/calix[4]resorcinarene molar ratio of 3:1 exhibits the lowest miscibility. These monolayers are also characterized by compressibility modulus lower in comparison with other ratios (i.e. monolayer is most fluid). This suggests that the calix[4]

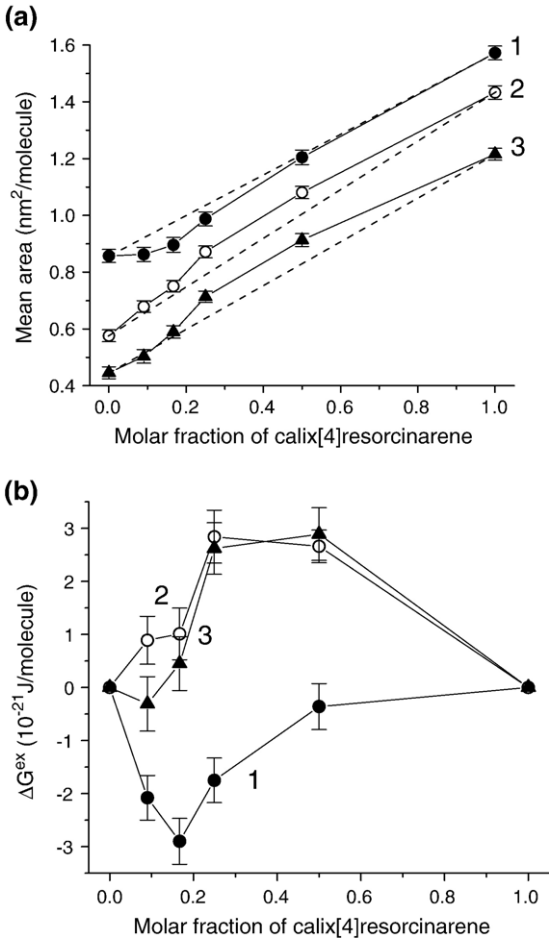

Fig. 9. The plot of (a) mean molecular area and (b) excess free energy, ΔG^{ex}, versus molar fraction of calix[4]resorcinarene in the DPPC monolayers calculated according to Eqs. (4) and (5), respectively for three values of surface pressures: ●—5; ○—15; ▲—35 mN/m. Monolayers were formed on water subphase contained 10^{-4} M dopamine. Results are mean±SD determined from 6 independent measurements.

resorcinarene molecules in these monolayers are oriented in such a way that minimizes the interaction with dopamine. This suggestion is also supported by small changes of the Gibbs free energy in the presence of dopamine (see Table 3). On the contrary, for the ratio 1:1 an increase of Gibbs energy from 1.89 to 2.94 kJ/mol (i.e. $\Delta G = 1.05$ kJ/mol, see Table 3) was observed when the monolayer was spread on the subphase contained 100 μM dopamine and compressed up to 35 mN/m. In this case the number of calix[4]resorcinarene molecules is enough to take the proper orientation that is capable to bind dopamine.

It is evident that further study including application of phospholipids composed of unsaturated hydrophobic chains is necessary to optimize the properties of the mixed calix[4] resorcinarene –phospholipids monolayers, especially for practical purpose of construction of affinity biosensors based on artificial receptors. Application of phospholipids could help in formation of calix[4]resorcinarene layers on a solid support covered by amphiphilic film and thus to protect the sensor from undesirable interferences with other, especially charged compounds. This is important particularly for development mass sensitive sensors using quartz crystal microbalance (QCM) [26] or optical sensors using surface plasmon resonance (SPR) [30] technique.

4. Conclusions

Calix[4]resorcinarene, DPPC and their mixtures form stable monolayers at the water subphase. Analysis of the miscibility revealed that individual components are weakly or not miscible at the range of the molar ratios investigated and mostly form aggregates containing the molecules of only one type. The unfavorable interactions between the molecules of the both types was proved by the positive values of the Gibbs energies of mixing, mainly at the higher surface pressure, when the monolayers are in solid state. Presence of dopamine resulted in increase of the mean molecular areas and in destabilization of the monolayers. This was suggested on the base of lowering of the compressibility modulus of monolayers and from positive values of Gibbs free energies of the monolayers formed on water subphase with and without dopamine. Destabilization of the monolayers may probably occur due to the binding of dopamine inside the calix[4]resorcinarene cavities and due to consecutive changes of the structure of the calix[4] resorcinarene. These effects probably cause the weakening of the forces between the molecules in the monolayer. Measurement of dipole potential provided useful information on the monolayer–dopamine complexation. Changes of the potential regarding to the pure water subphase depend on the ability of the monolayer to bind dopamine.

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